1979

Field and laboratory studies on suppressing populations of the European corn borer, Ostrinia nubilalis, with Nosema pyrausta, Bacillus thuringiensis, carbaryl and carbofuran

John Lublinkhof
Iowa State University

Field and laboratory studies on suppressing populations of the European corn borer, *Ostrinia nubilalis*, with *Nosema pyrausta*, *Bacillus thuringiensis*, carbaryl and carbofuran

by

John Lublinkhof

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major: Entomology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1979

TABLE OF CONTENTS

INTRODUCTION

The European corn borer, Ostrinia nubilalis (Hübner), has been a major pest of corn in North America since its introduction to Massachusetts in 1917 (Vinal, 1917). Two generations per year of this pest occur in Iowa. First generation larvae primarily establish and survive on whorl stage corn causing extensive leaf damage until they tunnel into the corn stalk. Second generation larvae primarily establish and survive on the sheath-collar region of the corn plant before entering the corn stalk. Because of this life cycle, timing and type of insecticide application has always been of critical importance when attempting to suppress the population.

The increasing restrictions placed on insecticide use by the United States Environmental Protection Agency have directed researchers to investigate the possibility of using alternative methods of control or combining these methods with reduced rates of insecticides. The purpose of this dissertation is to examine the potential of Nosema pyrausta Paillot, a protozoan intracellular parasite of the European corn borer, when used in combination with Bacillus thuringiensis Berliner and two carbamate insecticides, carbaryl and carbofuran to suppress populations of the European corn borer. Such studies were carried out both in field and laboratory. The field investigations were designed to measure the effectiveness of N. pyrausta as a microbial insecticide when used by itself and in combination with other insecticides. Laboratory studies were designed

to measure the infectivity of N. pyrausta over time under field and laboratory conditions.

Overall, this research is an initial attempt to integrate the use of a pathogen in combination with a reduced rate of insecticide to suppress populations of the European corn borer.

REVIEW OF LITERATURE

European Corn Borer

Research developments on the biology, ecology and control of the European corn borer have been extensively reviewed by Brindley and Dicke (1963) and recently updated by Brindley et al. (1975). Therefore, only the pertinent research on suppressing populations of the European corn borer with N. pyrausta, B. thuringiensis, carbaryl and carbofuran will be reviewed here.

Nosema pyrausta

N. pyrausta was first isolated from the European corn borer by Paillot in 1927 (Paillot, 1928). Steinhaus (1951) reported the isolation of N. pyrausta from the European corn borer in the United States.

N. pyrausta is an obligatory intracellular protozoan parasite of the European corn borer and reduces egg hatch and larval development rate, fecundity and adult longevity (Zimmack et al., 1954; Zimmack and Brindley, 1957; Kramer, 1959a; Van Denburgh and Burbutis, 1962; Lewis et al., 1971; Windels et al., 1976). This microsporidium is the most effective of naturally occurring pathogens of the European corn borer according to Lewis and Lynch (1978).

Transmission of N. pyrausta infection occurs transovarially (Zimmack et al., 1954; Zimmack and Brindley, 1957; Kramer, 1959a). Histological examination revealed that N. pyrausta spores passed from the region of infected nurse cells into the developing oocyte (Zimmack and Brindley, 1957). Zimmack et al. (1954) further found that this infection can be

transmitted through an infected food source. Lewis (1978) reported that N. pyrausta can be disseminated via the frass. Zimmack and Brindley (1957) also observed that N. pyrausta spores could be found in all developmental stages of the European corn borer. These researchers noted further that all stages of N. pyrausta were found in the following European corn borer structures: Malpighian tubules of larvae and adults, silk glands of larvae and the genital tract of adult females.

According to Weiser (1963), an infection occurs when a microsporidium spore extrudes a polar filament while in the host's gut. This filament penetrates the peritrophic membrane and the gut epithelium and inserts sporoplasm into the gut wall which in turn is capable of migrating into the hemolymph and invading different tissues of the host. Kramer (1959b) observed this mechanism of infection for N. pyrausta in the European corn borer. Also, while studying the European corn borer, Zimmack and Brindley (1957) observed that the cytoplasm in the Malpighian tubules was replaced by N. pyrausta spores. These researchers further indicated that the pressure of N. pyrausta in the cytoplasm caused lesions and then allowed numerous spores to enter the lumen of the Malpighian tubules. The Malpighian tubules were the main site of infection in the European corn borer (Hall, 1952; Zimmack and Brindley, 1957; Kramer, 1959a).

N. pyrausta needs to be propagated in a living system and is, therefore, difficult to produce in large quantities (McLaughlin, 1971; Ignoffo and Hink, 1971). The lack of an in vitro production system for microsporida has allowed only limited research on the use of these

organisms as microbial insecticides. Zimmack et al. (1954) applied a spore suspension of N. pyrausta to corn plants and observed that N. pyrausta-free European corn borer larvae were able to pick up the infection but indicated that these results were inconclusive. Lewis and Lynch (1978) designed a field experiment testing the effectiveness of an aqueous suspension of vacuum-dried N. pyrausta spores on suppressing populations of the European corn borer on corn. These researchers indicated that an average of 63.8 and 97.2% of N. pyrausta-free European corn borer larvae in the field tests were capable of picking up the N. pyrausta infection in simulated first and second generation populations of the European corn borer, respectively. These infections resulted in a reduction in larvae and corn stalk cavities for both generations of the European corn borer. Henry (1971) formulated a wheat bran bait from Nosema locustae Canning obtained from laboratory reared grasshoppers. The bait was then applied at a rate of 2.24 kg/ha for control of rangeland grasshoppers. Henry indicated that application of N. locustae at as low as 7.75 spores/cm^2 on short grass vegetation in Montana was capable of infecting 33.7% of the combined species of grasshoppers compared to 3.9% in the control at six weeks post-treatment. He further indicated that 34.5% reduction of three combined species of grasshoppers was observed at six weeks post-application. The above workers demonstrated that microsporida have the potential of suppressing populations of pest insects in field situations.

Lewis (1975) indicated that European corn borer larvae are capable of tolerating a N. pyrausta infection under ideal conditions but that

under additional stress factors, populations can be suppressed substantially. Kramer (1959c) indicated that seasonal high and low temperatures are stress factors that interact with the N. pyrausta infection which causes mortality of European corn borer larvae. Weight and survival of 1st-generation European corn borer larvae were reduced when subjected to a combination of N. pyrausta infection and resistance to leaf feeding (Lewis and Lynch, 1976). Similarly, Lynch and Lewis (1976) reported that a N. pyrausta infection in combination with resistance in maize to sheath-collar feeding reduced the weight and survival of 2nd-generation European corn borer larvae. A combination of N. pyrausta infection with a stress factor such as insecticides has not been investigated. The results of studies examining a combination of N. pyrausta with B. thuringiensis, carbaryl and carbofuran for suppressing populations of the European corn borer in the field and laboratory will be reported in this dissertation.

Bacillus thuringiensis

B. thuringiensis was first isolated from the Mediterranean flour moth, Anagasta kühniella (Zeller) by Berliner in 1915 (Steinhaus, 1949). Subsequent investigators found that other insects, particularly in the Order Lepidoptera, were susceptible to B. thuringiensis and several varieties could be isolated (Heimpel, 1967). Heimpel (1967) critically reviewed the literature on B. thuringiensis var. thuringiensis and other crystalliferous bacteria. B. thuringiensis is not a very powerful contagion, but it has the characteristic of being very selective when used as a microbial insecticide (Burgerjon and Martouret, 1971). Another

desirable property is that it can be produced in large quantities on an aritificial medium. B. thuringiensis is produced commercially as a microbial insecticide and has been proven to be safe to man and other vertebrates (Heimpel, 1971).

The vegetative state of B. thuringiensis produces a spore, the resting form, which in turn forms a protein crystal body in the sporangium at the time of sporulation (Hannay, 1953). This protein crystal is referred to as a parasporal body, crystalline paraspore or δ-endotoxin. A combination of spores and crystals were necessary to cause mortality in European corn borer larvae (Sutter and Raun, 1966; Martouret and Anglade, 1971).

Sutter and Raun (1967) studied the histopathology of European corn borer larvae treated with B. thuringiensis and found that the crystalline paraspores induced gut paralysis and caused midgut epithelial cells to slough off into the lumen of the alimentary tract. After germination of the bacterial spores and the rapid buildup of vegetative rods, this bacterium penetrated the basement membrane. Invasion of the hemolymph with the gut contents causes mortality of the larva (Sutter and Raun, 1966; Sutter and Raun, 1967; Angus, 1968). Sutter and Raun (1967) found this mode of action to be the same in all larval instars.

An extensive amount of research has been conducted to determine the efficacy of B. thuringiensis. Satisfactory but variable results were obtained with B. thuringiensis granules in suppressing populations of the European corn borer (Raun, 1963; Raun and Jackson, 1966; McWhorter et al., 1972; Lynch et al., 1977a). Such variability among these results

may be attributed in part to formulation or lack of standardization (Lynch et al., 1977a, 1977b).

Dulmage et al. (1971) proposed a standardized bioassay procedure for formulations of the δ-endotoxin of B. thuringiensis based on international units (IU) and using the cabbage looper, Trichoplusia ni (Hübner) as the test organism. In 1972, HD-1-S-1971 was adopted as the Primary U.S. Reference Standard and was assigned a potency of 18,000 IU/mg based on 1000 IU/mg assigned to the original international standard, E-61 (Dulmage, 1973, 1975). The original international standard, E-61, was developed by Burges (1967).

Lynch et al. (1977b) used comparative bioassay techniques to standardize commercial B. thuringiensis products, Dipel® and Thuricide HPC® for the European corn borer. The Primary United States Reference Standard, HD-1-S-1971, was used as the standard and assigned a potency of 18,000 IU/mg. The activity of commercial B. thuringiensis products varies between years, and it is therefore necessary to standardize these products in terms of potency so that more consistent results can be obtained and more meaningful comparisons can be made.

Chemical Insecticides

The significant developments in the use of chemical insecticides for suppressing populations of the European corn borer were reviewed most recently by Brindley et al. (1975). Only the pertinent literature and developments since 1975 will be discussed here.

At present, the most effective means of suppressing populations of the European corn borer are by chemical insecticides. Stockdale et al. (1978) recommended use of granular formulations of the following insecticides for suppression of the European corn borer in Iowa: carbofuran, fonofos, EPN and phorate. McWhorter et al. (1976) tested carbaryl, EPN and carbofuran and measured their effectiveness in terms of reducing corn stalk cavities caused by 1st-generation European corn borer larvae and indicated that granular formulations were significantly more effective than spray formulations. These researchers further indicated that granular formulations are probably more effective due to being protected from the environment when in the whorl of the corn plant.

Carbaryl and carbofuran are wide spectrum carbamate insecticides. These act as cholinesterase inhibitors and are readily capable of penetrating the insect cuticle and nerve sheath (Matsumura, 1975). The action of granular carbofuran was found to be systemic (Edwards and Berry, 1972) but was also demonstrated to be effective as a contact insecticide for over the row applications on corn for suppressing 1st-and 2nd-generation populations of the European corn borer (Berry et al., 1974). Carbofuran was shown to be very effective in suppressing populations of 1st-generation European corn borer larvae as indicated by reduced cavities (McWhorter et al., 1976). Similarly, Berry et al. (1978) found that carbofuran was very effective in suppressing populations of 2nd-generation European corn borer larvae when applied over the row in corn and then incorporated into the soil. Since carbofuran was generally effective in suppressing populations of the European corn borer, the

intent in this dissertation is to examine its effectiveness when used at reduced rates and in combination with N. pyrausta. Although variable results were obtained when carbaryl was tested for control of the European corn borer (Harding et al., 1968; Berry et al., 1972; Berry et al., 1974), it was selected with the intent of possibly being an effective insecticide when used in combination with N. pyrausta.

Smith (1970) indicated that chemical insecticides must be judged on the basis of positive values that can be achieved by them when compared to the negative values that may result from their use. Data from this dissertation will attempt to more fully reflect the positive aspects of chemical insecticides when they are used in combination with pathogens.

MATERIALS AND METHODS

Field Studies

The field investigations were designed to measure the effectiveness of N. pyrausta as a microbial insecticide when used by itself and in combination with other insecticides. A hybrid corn, A632 X H95, susceptible to both leaf and sheath-collar feeding by the European corn borer, was planted in rows 0.75 m apart on May 20, 1976 and May 10, 1977 at the USDA Corn Insects Research Unit, Ankeny, Iowa.

The field experiments were designed as split-split plots and replicated four times. The whole plots were the insecticide treatments and checks; the split plots were disease categories, presence or absence of N. pyrausta; and, the split-split plots were the time intervals. Each split plot consisted of two 15.25 m rows of corn separated from adjacent plots by a guard row. Corn plants near the center of each row were marked and artificially infested with two egg masses (free of N. pyrausta infection) per plant for three successive days to simulate populations of the European corn borer. Egg masses were produced in the laboratory by the modified technique of Guthrie et al. (1971). Infestations were made on July 4, 5, 6 and August 15, 16, 17 in 1976 and June 26, 27, 28 and July 28, 29, 30 in 1977 during the mid-whorl and pollen-shedding stages within each year, respectively.

The whole plots were: three rates of B. thuringiensis, 1.2, 2.4 and 3.6×10^{10} IU/ha; three rates of carbaryl, 0.8, 1.7 and 3.4 kg AI/ha; three rates of carbofuran, 0.3, 0.6 and 1.1 kg AI/ha; and three untreated

checks. Carbaryl and carbofuran rates were calibrated according to the active ingredient stated on the label. Application procedures were essentially the same as those reported by Berry et al. (1974). The potency of B. thuringiensis granules are variable, therefore this product was assayed against larvae of the European corn borer each year using standard bioassay techniques as outlined by Lynch et al. (1977b). The split plots were the disease categories; N. pyrausta was applied to the split plot and then not applied to the corresponding split plot within each whole plot. At one day post-infestation of European corn borer egg masses, an aqueous suspension of 1.2×10^7 spores in 15 ml distilled water was applied to the whorl of the plant for 1st-generation larvae and sprayed on the corn plants between three nodes above and three nodes below the ear with a back-pack sprayer for 2nd-generation larvae. To obtain N. pyrausta spores, larvae from an infected European corn borer colony were homogenized with a blender for 2 min and then filtered through two layers of muslin cloth and quantified using a hemacytometer. These applications of N. pyrausta spores were made one day prior to the insecticide applications. The time intervals post-application constituted the split-split plots.

Five corn plants were randomly selected from within each split plot of the selected whole plots at various intervals post-application and the number of larvae found were recorded, collected and then frozen until they were examined for the incidence of N. pyrausta. The selected whole plots included the three untreated checks, the high rate of B. thuringiensis, the medium rate of carbaryl and the low rate of carbofuran.

The time intervals post-application constituted the split-split plots. Larval counts were made at 4, 8 and 12 day intervals for the 1st- and 2nd-generation tests in 1976. In 1977, larval counts were made at 5, 8 and 12 day intervals for the 1st-generation tests and at 4, 9, 15 and 44 day intervals for the 2nd-generation tests.

The 20 infested plants remaining within each split plot were split in half from the tassel to the base of the plant and the cumulative lengths of stalk cavities caused by European corn borer larvae were recorded. Stalk cavities were recorded at ca. 40 days post-infestation or thereafter for each generation in 1976 and 1977.

To determine whether water alone affects European corn borer mortality, an additional experiment was designed as a split plot and replicated four times. Corn plants were artificially infested with two egg masses (free of N. pyrausta infection) per plant for three successive days; July 13, 14, 15 for simulated 1st-generation populations of the European corn borer in 1976 only. The whole plot treatments were N. pyrausta applied at 3.24×10^7 spores in 15 ml distilled water per plant, 15 ml distilled water per plant and an untreated check. Five corn plants were randomly selected within each whole plot at 4, 8 and 12 day intervals of post-treatment. The time intervals constituted the split plots. Stalk cavities were not recorded for this experiment.

An analysis of variance was used to analyze the results of the field studies.

Laboratory Studies

Determination of the percentage and intensity of Nosema pyrausta infections in European corn borer larvae under field conditions

A maximum of ten larvae from each split-split plot in the field studies were checked for N. pyrausta infections by a modified technique of Raun et al. (1960). This involved weighing the larva, adding a proportionate amount of distilled water at a rate of 0.1 ml for each 1 mg of larval wt and then homogenizing the larva in a 10 ml teflon pestel tissue grinder which was attached to an electric powered tissue grinder drive. This homogenate was vortex mixed and a loopful was then placed on one side of a double chambered improved Neubauer hemacytometer and covered with a cover slip. To determine the number of N. pyrausta spores, 20 smaller squares were examined; four in each corner and four in the center of the chamber. Each smaller square is 1/400 mm^2. By multiplying the number of spores counted in the 20 squares by 20, the intensity (spores/ µg larval tissue) was obtained.

The percentage of larvae infected with N. pyrausta from each split-split plot and the intensity of those infected were determined. Larvae collected from plots not receiving N. pyrausta spore application were checked for percentage infection and spore intensity but were not included in the statistical analyses. Therefore, the application of N. pyrausta with insecticides constituted the whole plots and the time intervals at which larval counts were made constituted the split plots.

Determination of percentage mortality and the percentage and intensity of Nosema pyrausta infections in European corn borer larvae under laboratory conditions

Bioassay techniques were used to determine the LC_{30} values of B. thuringiensis, carbaryl and carbofuran when tested against European corn borer larvae. These were required to set up the laboratory experiments to determine the percentage mortality and the percentage and intensity of N. pyrausta infections in European corn borer larvae under laboratory conditions.

B. thuringiensis, spore-crystal powder (HD-1 run no. IF-16 prepared by the USDA Cotton Insects Research Laboratory, Brownsville, Texas); carbaryl, 80% wettable powder; and, carbofuran, 95.6% technical grade were tested against neonate European corn borer larvae. Appropriate concentrations were determined by preliminary bioassay studies and then ten concentrations of each insecticide were prepared with each successive concentration being exactly one half of the previous concentration. B. thuringiensis material was diluted with phosphate buffer solution (Dulmage et al., 1971) and carbaryl and carbofuran materials were diluted with distilled water. A wheat germ diet (Lewis and Lynch, 1969) with an addition of mold inhibitors (4.8 g of methyl p-hydroxy-benzoate, 8.3 ml propionic acid and 0.8 ml phosphoric acid) was prepared and maintained at 50-54^{o}C in a temperature regulated dispensing kettle. Malt containers were filled to 225 ml with diet and 25 ml of the solution containing the insecticide was added and mixed for 2 min with a malt mixer. Then 5 ml of the mixture was dispensed into each of 50 plastic condiment cups. These were allowed to solidify at room temperature and a single larva

known to be free of a N. pyrausta infection was placed into each cup, and then the larvae were incubated at 27° C and 70% RH. Readings of larval mortality were made at the 8 day interval and Daum's (1970) revised probit analysis computer program was used to obtain the LC_{30} values (µg/ml) of these insecticides against neonate larvae.

The experiment to determine the effect of insecticides with N. pyrausta on the European corn borer was designed as a split plot and replicated four times. The whole plots were the treatments; B. thuringiensis with N. pyrausta, carbaryl with N. pyrausta, carbofuran with N. pyrausta and N. pyrausta without an insecticide serving as a treated check. The time interval post-topical application of N. pyrausta spores constituted the split plots. Identical procedures were used to prepare and dispense the artificial wheat germ diet as outlined for the above bioassay experiment. The quantity of insecticide incorporated into the diet was derived from the LC_{30} values in the above bioassay experiment and was equivalent to 3.05 µg of spore-crystal powder (HD-1 run no. IF-16)/ml, 5.17 µg AI/ml and 0.46 µg/AI/ml for B. thuringiensis, carbaryl and carbofuran, respectively. Twenty condiment cups were prepared for each treatment and allowed to solidify at room temperature and were then stored in a 4.4°C refrigerator for 24 hr. N. pyrausta spores were then topically applied at 1000 spores/mm^2 diet surface in 0.2 ml of distilled water. Prior to this, egg masses from a laboratory colony were heat treated in a distilled water bath at 43.3°C for 30 min (Raun, 1961) to assure N. pyrausta-free larvae for experimentation. After removing the condiment cups from the refrigerator, four N. pyrausta-free neonate larvae

were placed on diet in each condiment cup and then incubated at 27°C and 70% RH. Four condiment cups each containing four larvae were randomly selected within each whole plot at 3, 6, 8, 10 and 14 day intervals post-topical application of N. pyrausta spores and were then frozen until further examination. The time intervals constituted the split plots. Percentage larval mortality and the percentage of larvae infected were determined for all time intervals. The intensity of the N. pyrausta infection was determined at the 8, 10 and 14 day intervals only, using the same technique as in the previous section. Larvae were too small for accurate intensity determinations at the 3 and 6 day intervals but were examined for presence or absence of spores.

An analysis of variance was used to analyze the results of the laboratory studies.

RESULTS AND DISCUSSION

Field Studies

Larval counts

The larval counts for the whole plots (insecticide treatments) are presented in Table 1. During the 1st-generation, 1976, there were significantly fewer larvae on the plants receiving carbaryl but this was not significantly different from the B. thuringiensis treatments. There were significantly more larvae on the plants receiving carbofuran than on those treated with B. thuringiensis. All insecticide treated plants had significantly fewer larvae than the check. In the 2nd-generation, 1976, there were fewer larvae on the plants treated with carbaryl but this value was not significantly different from those treated with B. thuringiensis and carbofuran. There were significantly fewer larvae in the B. thuringiensis and carbaryl treatments than in the check. In 1977, during both generations, there were significantly fewer larvae in the B. thuringiensis treatments than in all other treatments. Fewer larvae were found on the carbaryl treated plants than on the carbofuran treated plants but these values did not differ significantly from each other. In both generations, there were significantly more larvae on the check plants than on the insecticide treated plants. At these rates, carbaryl and B. thuringiensis were most effective and carbofuran was least effective in reducing the number of larvae per plant.

The number of larvae per plant for the split plots (disease categories) are presented in Table 2. In both generations of 1976 and 1977,

Table 1. Effect of granular insecticides on European corn borer larvae (larval counts) summed over disease categories and days. Ankeny, Iowa, 1976 and 1977

Treatment	Rate/ha		Mean no. larvae/plant[1]			
			1976		1977	
	IU x 10^{10}	Kg AI	1st Generation[2,3]	2nd Generation[2,3]	1st Generation[2,3]	2nd Generation[2,4]
B. thuringiensis[5]	3.56		1.3 c	8.8 b	2.2 c	2.1 c
Carbaryl		1.68	1.2 c	8.1 b	4.4 b	2.9 b
Carbofuran		0.28	2.4 b	10.1 ab	4.6 b	3.1 b
Check			4.6 a	11.1 a	7.2 a	4.0 a

[1] Based on 5 plants per split-split plot within each replication.

[2] Means within columns followed by the same letter are not significantly different at the 0.05 level as indicated by Duncan's Multiple Range Test.

[3] Means within columns are based on 24 values except the checks are based on 72 values.

[4] Means within columns are based on 32 values except the check is based on 96 values.

[5] 200m = 4.41 x 10^{8} IU/kg.

Table 2. Effect of Nosema pyrausta application on European corn borer larvae (larval counts) summed over insecticide treatments and days. Ankeny, Iowa, 1976 and 1977

Application	Rate/plant Spores x 10^7	Mean no. larvae/plant[1] 1976 1st Generation[2,3]	1976 2nd Generation[2,3]	1977 1st Generation[2,3]	1977 2nd Generation[2,4]
With N. pyrausta	1.2	2.0 b	9.1 b	4.5 b	3.0 b
Without N. pyrausta	0.0	4.2 a	11.0 a	6.3 a	3.6 a

[1] Based on 5 plants per split-split plot within each replication.
[2] Means within columns followed by the same letter are not significantly different at the 0.05 level as indicated by the analysis of variance.
[3] Means within columns are based on 72 values.
[4] Means within colums are based on 96 values.

plots receiving an application of N. pyrausta had significantly fewer larvae per plant than those not receiving the application. These results agree with those of Lewis and Lynch (1978) who found that the application of N. pyrausta spores reduced the number of 1st-generation larvae by 48.4, 18.8 and 43.8%, for three separate tests. These researchers also reported that 2nd-generation larvae were reduced by 17.2 and 14.1%, for two separate tests when N. pyrausta spores were applied.

A significant insecticide treatment by disease category interaction was noted in the 1st-generation of 1976. However, these interactions were not significant in the 2nd-generation of 1976. Also, there were no significant insecticide treatment by disease category interactions in 1977, indicating that the insecticide treatments and the N. pyrausta acted independently and their effects in terms of reducing larvae were additive. Mussgnug and Henry (1979) also found that interactions between malathion and N. locustae were not evident in terms of reducing the migratory grasshopper, Melanoplus sanguinipes (F.) and indicated that each acted independently and that their effects were additive.

The larval count comparisons for the split-split plots (days) are presented in Table 3. In the 1st-generation of 1976, the number of larvae per plant decreased over a 12 day period. However, no significant difference in larval counts were noted over days in the 1st-generation of 1977. In the 2nd-generation of 1976 and 1977, differences are more variable over time. These data indicate that the greatest larval reduction occurred shortly after insecticide and N. pyrausta applications were made.

Table 3. Effect of time post-treatment of insecticides on European corn borer larvae (larval counts) summed over treatments and disease categories. Ankeny, Iowa, 1976 and 1977

Mean no. larvae/plant[1]							
1976				1977			
Day	1st Generation[2,4]	Day	2nd Generation[2,4]	Day	1st Generation[3,4]	Day	2nd Generation[2,4]
4	4.2 a	4	9.0 b	5	5.3 a	4	2.6 c
8	3.0 b	8	11.3 a	8	5.8 a	9	3.5 b
12	2.2 c	12	10.0 b	12	5.2 a	15	4.4 a
						44	2.9 c

[1]Based on 5 plants per split-split plot within each replication.

[2]Means within columns followed by the same letter are not significantly different at the 0.05 level as indicated by Duncan's Multiple Range Test.

[3]Means within columns followed by the same letter are not significantly different at the 0.05 level as indicated by the analysis of variance.

[4]Means within columns are based on 48 values.

The insecticide treatment by day interaction was significant in the 1st-generation of 1976 but the interactions were not significant for the 2nd-generation of 1976 and for both generations of 1977. There were not significant disease category by day interactions in either generation of 1976 and 1977 and this suggests that any larval reduction due to N. pyrausta occurred shortly after the application of this microsporidium. There were no significant insecticide treatment by disease category by day interactions during 1976 and 1977.

Table 4 presents larval count comparisons for the whole plots (treatments) in the experiment to determine whether water alone affects larval mortality. The check and distilled water (DH_2O) only treatments had an equal number of larvae per plant, both being significantly greater than the N. pyrausta treatment. The results reported by Lewis and Lynch (1978) also indicated that the amount of water applied had no effect on reducing the number of larvae per plant in their N. pyrausta spore application experiments. The number of larvae per plant did not differ significantly among days (Table 5) suggesting that most of the larval reduction occurred shortly after application of N. pyrausta. This is in agreement with the results of the previous field experiments.

Stalk cavity counts

The number of stalk cavities per plant for the whole plots (insecticide treatments) are presented in Table 6. In the 1st-generation of 1976, the high rate of carbofuran treatment had the fewest number of cavities per plant but this value did not differ significantly from those

Table 4. Effect of Nosema pyrausta and distilled water (DH_2O) applications on European corn borer larvae (larval counts) summed over days. Ankeny, Iowa, 1976

Treatment	Larvae/plant[1]
3.2×10^7 spores N. pyrausta in 15 ml DH_2O	3.1 b
15 ml DH_2O	5.0 a
Check	5.1 a

[1]Based on 5 plants per split plot within each replication. Means within columns followed by the same letter are not significantly different at the 0.05 level as indicated by Duncan's Multiple Range Test. Means within columns are based on 12 values.

Table 5. Effect of time post-application of Nosema pyrausta and distilled water on European corn borer larvae (larval counts) summed over applications. Ankeny, Iowa 1976

Day	Mean no. larvae/plant[1]
4	5.2 a
8	4.1 a
12	3.9 a

[1]Based on 5 plants per split plot within each replication. Means within columns followed by the same letter are not significantly different at the 0.05 level as indicated by the analysis of variance. Means within columns are based on 12 values.

Table 6. Effect of granular insecticides on European corn borer larvae (stalk cavity counts) summed over disease categories. Ankeny, Iowa, 1976 and 1977

Treatment	Rate/ha IU x 10^{10}	Rate/ha Kg AI	Mean cm damage/plant[1] 1976 1st Generation[2]	1976 2nd Generation[2]	1977 1st Generation[2]	1977 2nd Generation[2]
B. thuringiensis[3]	1.19		3.2 b	14.1 bc	3.4 bcd	7.2 ab
	2.37		2.6 bc	13.5 bc	3.5 bcd	8.6 ab
	3.56		2.1 cde	12.2 bc	3.2 cd	5.7 b
Carbaryl		0.84	2.2 bcd	15.1 ab	4.5 abc	10.1 a
		1.68	1.9 cde	11.8 bc	5.3 ab	8.8 ab
		3.36	1.3 de	11.7 bc	3.4 bcd	9.7 a
Carbofuran		0.28	2.7 bc	12.6 bc	4.4 bc	10.2 a
		0.56	1.3 de	12.6 bc	3.0 cd	8.8 ab
		1.12	1.1 e	10.8 c	2.2 d	8.6 ab
Check			6.1 a	17.0 a	6.0 a	9.9 a

[1]Based on 20 plants per split plot within each replication.

[2]Means within columns followed by the same letter are not significantly different at the 0.05 level as indicated by Duncan's Multiple Range Test. Means within columns are based on 8 values except the checks are based on 24 values.

[3]200m = 4.41 x 10^{8} IU/kg.

of the medium rate of carbofuran, high rate of B. thuringiensis and the medium and high rates of carbaryl. Stalk cavity counts for the check were significantly higher than all insecticide treatments. During the 2nd-generation of 1976, the number of stalk cavities per plant were the greatest for the check but this value was not significantly different from the low rate of carbaryl. The high rate of carbofuran treatment was most effective in contributing to the reduction of stalk cavities although these cavity counts were not significantly different from those in all other insecticide treatments except for the low rate of carbaryl. During the 1st-generation of 1977, the stalk cavity counts were the greatest in the check but these counts did not differ significantly from those in the low or medium rates of carbaryl. Stalk cavity counts were smallest for the high rate of carbofuran but this value did not differ significantly from the medium rate of carbofuran, the high rate of carbaryl and all rates of B. thuringiensis. In the 2nd-generation of 1977, stalk cavity counts in all insecticide treatments except the high rate of B. thuringiensis did not differ significantly from the check. Data for the 2nd-generation of 1977 are quite variable and may be a result of the abnormally dry and wet months of July and August, respectively. The data for both generations, 1976 and 1977 indicate that granular formulations of B. thuringiensis, carbaryl and carbofuran are all effective in contributing to the reduction of stalk cavities if a sufficient rate is used. These results agree with those of McWhorter et al. (1976) who reported that granular formulations of carbaryl and carbofuran at rates of 2.25 kg AI/ha

and 1.12 kg AI/ha, respectively, gave persistent reduction in stalk cavities caused by 1st-generation European corn borer larvae. The above results also agree with those of Lynch et al. (1977a) who demonstrated that granules of B. thuringiensis used at a rate of 2.24 kg AI/ha provided 48.7 and 43.4% reduction, respectively, in stalk cavities caused by 1st- and 2nd-generation European corn borer larvae.

The number of stalk cavities per plant for the split plots (disease categories) are presented in Table 7. In both generations of 1976 and 1977, corn plants receiving applications of N. pyrausta had significantly fewer stalk cavities than those not receiving the application. Such a reduction of stalk cavities was also reported in the spore application studies on European corn borer larvae conducted by Lewis and Lynch (1978). These researchers found that application of N. pyrausta spores reduced 1st-generation stalk cavities by 47.1, 42.9 and 39.3% in three separate tests. In two additional tests, these researchers also reported the reduction in stalk cavities caused by 2nd-generation European corn borer larvae.

A significant insecticide treatment by disease category interaction was noted in the 1st-generation of 1976. Interactions were not significant in the 2nd-generation of 1976 and in both generations of 1977, indicating that the insecticide treatments and N. pyrausta acted independently and their effects in terms of contributing to the reduction of stalk cavities were additive.

Table 7. Effect of *Nosema pyrausta* application on European corn borer larvae (stalk cavity counts) summed over insecticide treatments. Ankeny, Iowa, 1976 and 1977

Application	Rate/plant	Mean cm damage/plant[1]			
		1976		1977	
	Spores x 10^7	1st Generation[2]	2nd Generation[2]	1st Generation[2]	2nd Generation[2]
With *N. pyrausta*	1.2	1.7 b	12.0 b	3.9 b	8.3 b
Without *N. pyrausta*	0.0	4.4 a	15.5 a	4.6 a	9.6 a

[1]Based on 20 plants per split plot within each replication.

[2]Means within columns followed by the same letter are not significantly different at the 0.05 level as indicated by the analysis of variance. Means within columns are based on 48 values.

Laboratory Studies

Determination of the percentage and intensity of Nosema pyrausta infections in European corn borer larvae under field conditions

The percentage of European corn borer larvae obtaining an infection of N. pyrausta from the foliar application of this microsporidium for the whole plots (insecticide treatments) are presented in Table 8. Larvae collected from the split-split plots in the field experiments not receiving applications of N. pyrausta had a very low percentage of infection and therefore were not included in the statistical analyses. During both generations of 1976 and the 1st-generation of 1977, the percentage of larvae obtaining a N. pyrausta infection did not differ significantly among insecticide treatments and the check. In the 2nd-generation of 1977, only plots receiving carbofuran had a significantly lower percentage of larvae obtaining a N. pyrausta infection. These data suggest that insecticide treatments do not inhibit European corn borer larvae from obtaining a N. pyrausta infection. Maddox (1977) reported that when Vairimorpha necatrix (Kramer) spores were exposed for 30 min to TEPP at concentrations of 0.0064 to 1.25% and to malathion at a 0.5% concentration, they were still capable of causing a 100% infection of 6th-stage larvae of the armyworm, Pseudaletia unipuncta (Haworth). Similarly, Mussgnug and Henry (1979) found that the percentage of infection of migratory grasshoppers by N. locustae was not affected when each grasshopper was treated with 0.4, 0.6 or 0.8 µg of malathion.

The percentages of larvae obtaining a N. pyrausta infection for the split plots (days) are presented in Table 9. In the 1st-generation of

Table 8. Effect of granular insecticides on the percentage of European corn borer larvae obtaining a *Nosema pyrausta* infection summed over days. Ankeny, Iowa, 1976 and 1977

Treatment	Rate/ha		% infected larvae			
			1976		1977	
	IU x 10^{10}	Kg AI	1st Generation[1,3]	2nd Generation[1,3]	1st Generation[1,3]	2nd Generation[2,4]
B. thuringiensis[5]	3.56		52 a	46 a	61 a	69 a
Carbaryl		1.68	53 a	54 a	77 a	72 a
Carbofuran		0.28	64 a	48 a	79 a	59 b
Check			63 a	53 a	70 a	66 a

[1]Means within columns followed by the same letter are not significantly different at the 0.05 level as indicated by the analysis of variance.

[2]Means within columns followed by the same letter are not significantly different at the 0.05 level as indicated by Duncan's Multiple Range Test.

[3]Means within columns are based on 12 values except the checks are based on 36 values.

[4]Means within columns are based on 16 values except the checks are based on 48 values.

[5]200m = 4.41 x 10^8 IU/kg.

Table 9. Effect of time post-treatment of insecticides on the percentage of European corn borer larvae obtaining a Nosema pyrausta infection summed over treatments. Ankeny, Iowa 1976 and 1977

% infected larvae							
1976				1977			
Day	1st Generation[1,3]	Day	2nd Generation[2,3]	Day	1st Generation[2,3]	Day	2nd Generation[2,3]
4	60 a	4	45 b	5	46 c	4	47 c
8	61 a	8	65 a	8	78 b	9	67 b
12	59 a	12	43 b	12	89 a	15	61 b
						44	91 a

[1]Means within columns followed by the same letter are not significantly different at the 0.05 level as indicated by the analysis of variance.

[2]Means within columns followed by the same letter are not significantly different at the 0.05 level as indicated by Duncan's Multiple Range Test.

[3]Means within columns are based on 24 values.

1976, the percentage of larvae obtaining an infection did not differ significantly among days. In the 2nd-generation of 1976, the percentage infection at the 8 day interval was significantly greater than those at either the 4 or 12 day interval which were not significantly different from each other. During both generations of 1977, a definite increase in the percentage of larvae obtaining a N. pyrausta infection was noted over time intervals of 12 and 44 days for each generation, respectively. The data in 1977 suggest that the N. pyrausta infection can be disseminated through oral transmission or via the frass in the field. These results agree with those of Lewis (1978) who found that N. pyrausta from infected larvae were disseminated to uninfected larvae via the frass which resulted in a high incidence and intensity of infection and reduced populations of the European corn borer. In addition, larvae may have been still consuming viable N. pyrausta spores over time. Maddox (1977) indicated that the longevity of microsporidan spores is dependent on a number of factors such as sunlight, temperature, humidity and type of substrate.

A significant insecticide treatment by day interaction was noted for the 1st-generation of 1976 and 1977. In 1976, the percentage of larvae obtaining a N. pyrausta infection for all insecticide treatments and the check at the 4 day interval was between 48.0 and 65.8%. These percentages increased to between 66.3 and 75.0% at the 8 day interval for all insecticide treatments and the check except for B. thuringiensis which decreased to 15.8%. Similarly, during 1977, the percentage of larvae obtaining a N. pyrausta infection for all insecticide treatments and the check at the 5 day interval was between 37.9 and 62.0%. These

percentages increased to between 80.0 and 87.5% at the 8 day interval for all insecticide treatments and the check except for B. thuringiensis which only increased to 48.8%. After the 8 day interval, the percentage of infected larvae increased rapidly in the B. thuringiensis plots reaching levels comparable to the other plots at the 12 day interval. The difference in reaction to the chemical insecticides versus the microbial insecticide is at this time unexplainable. However, insecticide treatment by day interactions were not significant for the 2nd-generation of 1976 and 1977.

The comparisons for the intensity (spores/μg larval tissue) of N. pyrausta infection in European corn borer larvae after application to the whole plots (insecticide treatments) are presented in Table 10. Statistical analyses of intensities were based on only those larvae obtaining a N. pyrausta infection. Larvae collected from the split plots that did not receive an application of N. pyrausta spores had a very low percentage of infection and low intensity and were not included in the statistical analyses. In both generations of 1976 and the 1st-generation of 1977, intensity did not differ significantly among insecticide treatments and the check. In the 2nd-generation of 1977, intensity was greatest for larvae collected from the carbaryl treated plots but this did not differ significantly from the check. Larvae from the B. thuringiensis treated plots had the least intensity but this did not differ significantly from larvae collected from the carbofuran treated plots or the check. These data indicate that insecticides do not significantly affect N. pyrausta

Table 10. Effect of granular insecticides on the intensity of *Nosema pyrausta* infections in European corn borer larvae summed over days. Ankeny, Iowa, 1976 and 1977

Treatment	Rate/ha IU x 10^{10}	Rate/ha Kg AI	Spores/μg infected larval tissue 1976 1st Generation[1,3]	1976 2nd Generation[1,3]	1977 1st Generation[1,3]	1977 2nd Generation[2,4]
B. thuringiensis[5]	3.56		179 a	46 a	170 a	62 b
Carbaryl		1.68	179 a	44 a	138 a	93 a
Carbofuran		0.28	301 a	48 a	156 a	63 b
Check			361 a	44 a	186 a	80 ab

[1]Means within columns followed by the same letter are not significantly different at the 0.05 level as indicated by the analysis of variance.

[2]Means within columns followed by the same letter are not significantly different at the 0.05 level as indicated by Duncan's Multiple Range Test.

[3]Means within columns are based on 12 values except the checks are based on 36 values.

[4]Means within columns are based on 16 values except the checks are based on 48 values.

[5]200m = 4.41 x 10^8 IU/kg.

intensity in European corn borer larvae when applied under the conditions of this experiment.

The comparisons for the intensity of N. pyrausta infection in European corn borer larvae after their application to the split plots (days) are presented in Table 11. In both generations of 1976 and 1977, there is an increase in intensity over time. During the 1st-generation of 1976 and 1977, intensity increased significantly over days. In the 2nd-generation of 1976, intensity also increased over days. However, intensity at the 8 and 12 day intervals were not significantly different from each other and intensity of the 4 and 8 day intervals did not differ significantly from each other. Again, in the 2nd-generation of 1977, the intensity increased significantly over days. It was greatest at the 44 day interval but this did not differ significantly from the 15 day interval. These data show that the intensity increases more rapidly over time in larvae during the 1st-generation as compared to larvae in the 2nd-generation. This may be partially due to application techniques or due to the morphology of the plant. N. pyrausta spores applied to the whorl are concentrated in a smaller area and since 1st-generation larvae feed here, they are initially capable of obtaining a larger dosage as compared to 2nd-generation larvae feeding in the sheath-collar region. Lewis (1978) also indicated that the intensity of a N. pyrausta infection is partially dependent on the quantity and viability of spores in the original inoculum. A significant insecticide treatment by day interaction was noted for the 2nd-generation of 1977 but not for either generation of 1976 or the 1st-generation of 1977.

Table 11. Effect of time post-treatment of insecticides on the intensity of Nosema pyrausta infections in European corn borer larvae summed over treatments. Ankeny, Iowa, 1976 and 1977

Spores/µg infected larval tissue							
1976				1977			
Day	1st Generation[1]	Day	2nd Generation[1]	Day	1st Generation[1]	Day	2nd Generation[1]
4	65 c	4	38 b	5	38 c	4	32 c
8	293 b	8	42 ab	8	169 b	9	65 b
12	512 a	12	54 a	12	305 a	15	95 a
						44	112 a

[1]Means within columns followed by the same letter are not significantly different at 0.05 level as indicated by Duncan's Multiple Range Test. Means within columns are based on 24 values.

Determination of the percentage mortality and the percentage and intensity of Nosema pyrausta infections in European corn borer larvae under laboratory conditions

The percentage and intensity of N. pyrausta infections in European corn borer larvae in combination with insecticides were determined under laboratory as well as field conditions.

Comparisons for the percentage larval mortality, the percentage of larvae obtaining a N. pyrausta infection and the intensity in the whole plots (treatments) are presented in Table 12. All insecticide treatments in combination with N. pyrausta had significantly greater percentage larval mortality than the N. pyrausta treatment alone. The percentage larval mortality was greatest for larvae receiving B. thuringiensis with N. pyrausta but did not differ significantly from larvae receiving carbaryl with N. pyrausta. The percentage larval mortality for larvae receiving carbofuran with N. pyrausta was smallest but this did not differ significantly from larvae receiving carbaryl with N. pyrausta. The percentage of larvae obtaining a N. pyrausta infection did not differ significantly among treatments. These results agree with those found in the field collected larvae. Maddox (1977) indicated that TEPP or malathion were compatible with V. necatrix spores and Mussgnug and Henry (1979) have shown malathion and N. locustae to be compatible in studies with migratory grasshoppers. The intensity of N. pyrausta infections was greatest in larvae feeding on diet topically treated with N. pyrausta only and differed significantly from larvae receiving the insecticide treatments. The intensity of infection for larvae receiving carbaryl with N. pyrausta did not differ significantly from those receiving carbofuran with N. pyrausta

Table 12. Effect of insecticides with *Nosema pyrausta* on European corn borer larvae (% mortality, % infection and intensity) summed over days. Ankeny, Iowa, 1978

Treatment	% larval mortality[1,3]	% larvae infected[2,3]	Spores/µg larval tissue[1,4]
B. thuringiensis + *N. pyrausta*	72 a	64 a	88 c
Carbaryl + *N. pyrausta*	64 ab	77 a	326 b
Carbofuran + *N. pyrausta*	58 b	74 a	269 b
N. pyrausta	44 c	85 a	518 a

[1]Means within columns followed by the same letter are not significantly different at the 0.05 level as indicated by Duncan's Multiple Range Test.
[2]Means within columns followed by the same letter are not significantly different at the 0.05 level as indicated by the analysis of variance.
[3]Means within columns are based on 20 values.
[4]Means within columns are based on 12 values.

but the intensity or infection in the larvae in both of these treatments was significantly greater than the intensities in those larvae receiving B. thuringiensis with N. pyrausta. These data suggest that carbaryl, carbofuran and B. thuringiensis may have an effect on intensity. Maddox (1977) found that TEPP at a concentration of 1.25% and exposure time of 60 min caused reduction in the spore viability of V. necatrix. This suggests that certain insecticides, depending on concentrations and exposure times, can have a toxic effect on microsporida. Very low intensity was noted for European corn borer larvae receiving B. thuringiensis with N. pyrausta but high mortality was also evident. Since only the survivors were examined, a true indication of actual spore intensity was not determined and a much higher intensity might have been present in the dead larvae. Also, Weiser (1963) indicated that microsporidan spores extrude a polar filament when in the host's gut, penetrate the peritrophic membrane and gut epithelium and insert sporoplasm into the hemolymph. This may provide an entrance to the hemocoel for B. thuringiensis and may account for the high larval mortality. As indicated in the field collected larvae, the percentage and the intensity of infection generally did not differ significantly among insecticide treatments. The differences in results may be attributed to the closer contact larvae in the laboratory had with N. pyrausta spores and insecticides.

Comparisons for percentage larval mortality, percentage of larvae obtaining a N. pyrausta infection and intensity of the infection for the split plots (days) are presented in Table 13. The percentage larval mortality did not differ significantly among the 8, 10 and 14 day

Table 13. Effect of time post-treatment of insecticides with Nosema pyrausta on European corn borer larvae (% mortality, % infection and intensity) summed over treatments. Ankeny, Iowa, 1978

Day	% larval mortality[1]	% larvae infected[1]	Spores/µg larval tissue[1]
3	27 c	37 b	-
6	59 b	82 a	-
8	71 a	80 a	152 c
10	69 a	87 a	325 b
14	71 a	88 a	424 a

[1]Means within columns followed by the same letter are not significantly different at the 0.05 level as indicated by Duncan's Multiple Range Test. Means within columns are based on 16 values.

intervals but differed significantly from the 6 day interval which in turn differed significantly from the percent mortality at the 3 day interval. These data suggest that the insecticide treatments with N. pyrausta had their greatest effect in terms of larval mortality prior to the 8 day interval. This also agrees with the field studies where larval reduction was the greatest shortly after the insecticide and N. pyrausta applications were made. The percentage of larvae obtaining a N. pyrausta infection did not differ significantly among the 6, 8, 10 and 14 day intervals but differed significantly from the 3 day interval. A large percentage of infected larvae were noted prior to 6 days but such high percentages were also noted prior to 12 and 44 days for 1st- and 2nd-generation larvae collected from the field. A significant increase in N. pyrausta intensity occurred over the 8, 10 and 14 day intervals. These results compare with those found in the field collected larvae. As indicated by Lewis (1978), N. pyrausta from infected larvae were disseminated to uninfected larvae via the frass and caused a high incidence and intensity of disease in populations of the European corn borer.

Treatment by day interactions for percentage mortality and intensity of infection were not significant but a significant interaction was evident in the percentage of larvae obtaining a N. pyrausta infection. Such an interaction was also evident in the field collected larvae for the 1st-generation of 1976 and 1977. Interaction data for all treatments indicate that the percentage of larvae obtaining a N. pyrausta infection was between 29.3 and 54.8% at the 3 day interval and increased to between 70.3 and 89.0% at the 6 day interval. These percentages increased to

between 86.8 and 97.0% at the 8 day interval for all treatments except B. thuringiensis with N. pyrausta which decreased to 50%. After the 8 day interval, the percentage infection increased rapidly in the B. thuringiensis with N. pyrausta treatments reaching levels comparable to the other treatments. This pattern was also evident for larvae collected in the field. The difference in reaction to the chemical insecticides versus the microbial insecticide is at this time unexplainable.

SUMMARY AND CONCLUSIONS

Objectives of this study were 1) to measure the effectiveness of N. pyrausta when used in combination with insecticides to suppress populations of the European corn borer in field and laboratory experiments and 2) to measure the infectivity of N. pyrausta over time in European corn borer larvae under seasonal field conditions and under laboratory conditions.

A split-split plot experimental design was used to evaluate larval counts from the field experiments. The whole plots were the insecticide treatments (B. thuringiensis, carbaryl, carbofuran) and the checks. The split plots were disease categories; N. pyrausta applied or N. pyrausta-free. The time intervals when larval counts were made constituted the split-split plots. These data indicated that the greatest larval reduction occurred shortly after application of the insecticides and N. pyrausta. The insecticides and N. pyrausta each acted independently and their effects in terms of reducing larval populations were additive.

For the field experiments, a split plot experimental design was used to evaluate the extent of stalk tunneling measured in terms of stalk cavity counts. The whole plots were the insecticide treatments and checks consisting of three untreated checks, three rates of B. thuringiensis, three rates of carbaryl and three rates of carbofuran. The split plots were disease categories: N. pyrausta applied or N. pyrausta-free. Data indicated that all insecticide treatments were effective in

contributing to the reduction of stalk cavities if a sufficient rate was used. The insecticides and N. pyrausta each acted independently and their effects in terms of reducing stalk cavities were additive.

From the field experiments, the percentage of larvae infected with N. pyrausta and the intensity of those infected were determined and statistically analyzed as a split plot experimental design. Insecticide treatments and checks with N. pyrausta application constituted the whole plots and time intervals constituted the split plots. The percentage of larvae establishing a N. pyrausta infection and the intensity did not differ significantly among insecticide treatments and the check. This suggests that insecticide treatments do not significantly affect the percentage of larvae obtaining a N. pyrausta infection nor the intensity of infection. Data indicated that intensity increased more rapidly over time for the 1st-generations as compared to the 2nd-generation in 1976 and 1977. This may be attributed to the N. pyrausta application techniques. Larvae may initially pick up larger dosages of N. pyrausta when the microsporidium is placed in the whorl as compared to being sprayed on the ear region of the plant. For the larval counts, the disease category by day interaction was not significant in both generations, 1976 and 1977. These data indicate that larval reduction occurred shortly after N. pyrausta application and suggest that larval reduction was dependent on an infection of N. pyrausta but not dependent on the intensity of infection.

A split plot experimental design was used to evaluate percentage mortality, percentage larval infection and intensity of infection in

European corn borer larvae under laboratory conditions. The whole plots were the treatments; B. thuringiensis with N. pyrausta, carbaryl with N. pyrausta, carbofuran with N. pyrausta and N. pyrausta only. The time intervals post-topical application of N. pyrausta spores constituted the split plots. As expected, percentage mortality was significantly greater in those treatments containing insecticides. The percentage of larvae obtaining a N. pyrausta infection was not significantly different among treatments suggesting that the rates of insecticides used do not affect the percentage of larvae obtaining a N. pyrausta infection. This agrees with the results from the field collected larvae. Insecticide treatments significantly decreased the intensity of infections which does not agree with the results of the field experiments but this is possibly due to the closer contact larvae in the laboratory had with N. pyrausta spores and insecticides.

Overall, N. pyrausta stresses populations of the European corn borer. If N. pyrausta could be established in a population as a result of their application, it is evident that this effect in combination with insecticide treatments could provide a very effective pest management strategy for suppressing populations of the European corn borer.

LITERATURE CITED

Angus, T. A. 1968. The use of Bacillus thuringiensis as a microbial insecticide. World Rev. Pest Control 7: 11-26.

Berry, E. C., J. E. Campbell, C. R. Edwards, J. A. Harding, W. G. Lovely and G. M. McWhorter. 1972. Further field tests of chemicals for control of the European corn borer. J. Econ. Entomol. 65: 1113-6.

Berry, E. C., J. F. Robinson, W. G. Lovely and G. M. McWhorter. 1974. European corn borer: Field trials with insecticides. Proc. North Cent. Branch Entomol. Soc. Am. 29: 128-31.

Berry, E. C., G. M. Ghidiu and W. D. Guthrie. 1978. European corn borer: Relationship of carbofuran uptake and corn genotypes for second generation control. Iowa State J. Research 53: 35-9.

Brindley, T. A. and F. F. Dicke. 1963. Significant developments in European corn borer research. Annu. Rev. Entomol. 8: 155-76.

Brindley, T. A., A. N. Sparks, W. B. Showers and W. D. Guthrie. 1975. Recent research advances on the European corn borer in North America. Annu. Rev. Entomol. 20: 221-39.

Burgerjon, A. and D. Martouret. 1971. Determination and significance of the host spectrum of Bacillus thuringiensis. Pages 305-25 in H. D. Burges and N. W. Hussey, eds. Microbial control of insects and mites. Academic Press, New York.

Burges, H. D. 1967. The standardization of products based on Bacillus thuringiensis. Pages 306-14 in P. A. Van Der Laan, ed. Insect pathology and microbial control: Proceedings of the International Colloquim on Insect Pathology and Microbial Control, Wageningen, The Netherlands, September 5-10, 1966. North-Holland Publishing Co., Amsterdam.

Daum, R. J. 1970. A revision of two computer programs for probit analysis. Bull. Entomol. Soc. Am. 16: 10-5.

Dulmage, H. T. 1973. B. thuringiensis U. S. assay standard. Report on the adoption of a primary U. S. reference standard for assay of formulations containing the δ-endotoxin of Bacillus thuringiensis. Bull. Entomol. Soc. Am. 19: 200-2.

Dulmage, H. T. 1975. The standardization of formulations of the δ-endotoxins produced by Bacillus thuringiensis. J. Invertebr. Pathol. 25: 279-81.

Dulmage, H. T., O. P. Boening, C. S. Rehnborg and G. D. Hansen. 1971. A proposed standardized bioassay for formulations of Bacillus thuringiensis based on the international unit. J. Invertebr. Pathol. 18: 240-5.

Edwards, C. R. and E. C. Berry. 1972. Evaluation of five systemic insecticides for control of the European corn borer. J. Econ. Entomol. 65: 1129-32.

Guthrie, W. D., W. A. Russel and C. W. Jennings. 1971. Resistance of maize to second-brood European corn borers. In Proceedings of the American Seed Trade Association. Annual Corn and Sorghum Research Conference 26: 65-79.

Hall, I. M. 1952. Observations on Perezia pyraustae Paillot, a microsporidian parasite of the European corn borer. J. Parasitol. 38: 48-52.

Hannay, C. L. 1953. Crystalline inclusions in aerobic sporeforming bacteria. Nature 172: 1004.

Harding, J. A., W. G. Lovely and R. C. Dyar. 1968. Field tests of chemicals for control of the European corn borer. J. Econ. Entomol. 61: 1427-30.

Heimpel, A. M. 1967. A critical review of Bacillus thuringiensis var. thuringiensis Berliner and other crystalliferous bacteria. Annu. Rev. Entomol. 12: 287-322.

Heimpel, A. M. 1971. Safety of insect pathogens for man and vertebrates. Pages 469-89 in H. D. Burges and N. W. Hussey, eds. Microbial control of insects and mites. Academic Press, New York.

Henry, J. E. 1971. Experimental application of Nosema locustae for control of grasshoppers. J. Invertebr. Pathol. 18: 389-94.

Ignoffo, C. M. and W. F. Hink. 1971. Propagation of arthropod pathogens in living systems. Pages 541-80 in H. D. Burges and N. W. Hussey, eds. Microbial control of insects and mites. Academic Press, New York.

Kramer, J. P. 1959a. Some relationships between Perezia pyraustae Paillot (Sporozoa, Nosematidae) and Pyrausta nubilalis (Hübner) (Lepidoptera, Pyralidae). J. Insect Pathol. 1: 25-33.

Kramer, J. P. 1959b. Studies on the morphology and life history of Perezia pyraustae Paillot (Microsporidia: Nosematidae). Trans. Am. Microsc. Soc. 78: 336-42.

Kramer, J. P. 1959c. Observations on the seasonal incidence of microsporidiosis in European corn borer populations in Illinois. Entomophaga 4: 37-42.

Lewis, L. C. 1975. Natural regulation of crop pests in their indigenous ecosystems and in Iowa agroecosystems: Bioregulation of economic insect pests. Iowa State J. of Research 49: 435-45.

Lewis, L. C. 1978. Migration of larvae of Ostrinia nubilalis (Lepidoptera: Pyralidae) infected with Nosema pyrausta (Microsporida: Nosematidae) and subsequent dissemination of this microsporidium. Can. Entomol. 110: 897-900.

Lewis, L. C. and R. E. Lynch. 1969. Rearing the European corn borer, Ostrinia nubilalis (Hübner), on diets containing corn leaf and wheat germ. Iowa State J. Sci. 44: 9-14.

Lewis, L. C. and R. E. Lynch. 1976. Influence on the European corn borer of Nosema pyrausta and resistance in maize to leaf feeding. Environ. Entomol. 5: 139-42.

Lewis, L. C. and R. E. Lynch. 1978. Foliar application of Nosema pyrausta for suppression of populations of European corn borer. Entomophaga 23: 83-8.

Lewis, L. C., R. E. Lynch and W. D. Guthrie. 1971. Biology of European corn borers reared continuously on a diet containing Fumadil B. Ann. Entomol. Soc. Am. 64: 1264-9.

Lynch, R. E. and L. C. Lewis. 1976. Influence on the European corn borer of Nosema pyrausta and resistance in maize to sheath-collar feeding. Environ. Entomol. 5: 143-6.

Lynch, R. E., L. C. Lewis, E. C. Berry and J. F. Robinson. 1977a. European corn borer: granular formulations of Bacillus thuringiensis for control. J. Econ. Entomol. 70: 389-91.

Lynch, R. E., L. C. Lewis, E. C. Berry and J. F. Robinson, 1977b. European corn borer control with Bacillus thuringiensis standardized as corn borer international units. J. Invertebr. Pathol. 30: 169-74.

Maddox, J. V. 1977. Stability of entomopathogenic protozoa. Misc. Publ. Entomol. Soc. Am. 10: 3-18.

Martouret, D. and P. Anglade. 1971. Bacillus thuringiensis in the control of corn borer Ostrinia nubilalis Hübn. (In French, English summary). Annales de Zoologie Ecologie Animale 3:57-58.

Matsumura, F. 1975. Toxicology of insecticides. Plenum Press, New York.

McLaughlin, R. E. 1971. Use of protozoans for microbial control of insects. Pages 151-72 in H. D. Burges and N. W. Hussey, eds. Microbial control of insects and mites. Academic Press, New York.

McWhorter, G. M., E. C. Berry and L. C. Lewis. 1972. Control of the European corn borer with two varieties of Bacillus thuringiensis. J. Econ. Entomol. 65: 1414-7.

McWhorter, G. M., E. C. Berry and J. F. Robinson. 1976. Field persistence of six insecticides for European corn borer control. J. Econ. Entomol. 69: 419-20.

Mussgnug, G. M. and J. E. Henry. 1979. Compatibility of malathion and Nosema locustae (Microsporidia: Nosematidae) in Melanoplus sanguinipes. Acrida (In press).

Paillot, A. 1928. On the natural equilibrium of Pyrausta nubilalis Hb. Pages 77-106 in T. U. Ellinger, ed. International Corn Borer Investigations, Scientific Reports 1927-1928.

Raun, E. S. 1961. Elimination of microsporidiosis in laboratory-reared European corn borers by the use of heat. J. Insect Pathol. 3: 446-8.

Raun, E. S. 1963. Corn borer control with Bacillus thuringiensis Berliner. Iowa State J. Sci. 38: 141-50.

Raun, E. S. and R. D. Jackson. 1966. Encapsulation as a technique for formulating microbial and chemical insecticides. J. Econ. Entomol. 59: 620-2.

Raun, E. S., G. T. York and D. L. Brooks. 1960. Determination of Perezia pyraustae infection rates in larvae of the European corn borer. J. Insect Pathol. 2: 254-8.

Smith, R. F. 1970. Pesticides: their use and limitations in pest management. Pages 103-13 in R. L. Rabb and F. E. Guthrie, eds. Concepts of pest management: Proceedings of a Conference held at North Carolina State Univ. at Raleigh, Raleigh, North Carolina, March 25-27, 1970.

Steinhaus, E. A. 1949. Principles of insect pathology. McGraw-Hill Book Company, Inc., New York.

Steinhaus, E. A. 1951. Report on diagnoses of diseased insects 1944-1950. Hilgardia 20: 629-78.

Stockdale, H. J., J. R. DeWitt and D. R. Lewis. 1978. Summary of Iowa pest control recommendations for 1978. Iowa Cooperative Extension Service IC-328 (Rev.).

Sutter, G. R. and E. S. Raun. 1966. The effect of Bacillus thuringiensis components on the development of the European corn borer. J. Invertebr. Pathol. 8: 457-60.

Sutter, G. R. and E. S. Raun. 1967. Histopathology of European corn borer larvae treated with Bacillus thuringiensis. J. Invertebr. Pathol. 9: 90-103.

Van Denburgh, R. S. and P. O. Burbutis. 1962. The host-parasite relationship of the European corn borer, Ostrinia nubilalis and the protozoan, Perezia pyraustae in Delaware. J. Econ. Entomol. 55: 65-7.

Vinal, S. C. 1917. The European corn borer, Pyrausta nubilalis Hübner, a recently established pest in Massachusetts. Mass. Agric. Exper. Sta. Bull. 178: 147-52.

Weiser, J. 1963. Sporozoan infections. Pages 291-334 in E. A. Steinhaus, ed. Insect pathology, an advanced treatise. Vol. 2. Academic Press, New York.

Windels, M. B., H. C. Chiang and B. Furgala. 1976. Effects of Nosema pyrausta on pupa and adult stages of the European corn borer Ostrinia nubilalis. J. Invertebr. Pathol. 27: 239-42.

Zimmack, H. L. and T. A. Brindley. 1957. The effect of the protozoan parasite Perezia pyraustae Paillot on the European corn borer. J. Econ. Entomol. 50: 637-40.

Zimmack, H. L., K. D. Arbuthnot and T. A. Brindley. 1954. Distribution of the European corn borer parasite Perezia pyraustae, and its effect on the host. J. Econ. Entomol. 47: 641-5.

ACKNOWLEDGMENTS

A very special thanks is extended to Dr. Leslie C. Lewis, my major professor, for his interest and guidance in my Ph.D. program. High appreciation and regards go to Dr. David F. Cox who has counseled me in the statistical analyses of these research data. The author would also like to thank Dr. Wilbur D. Guthrie, Dr. Jon J. Tollefson and Dr. Donald G. Woolley for serving on the program of study committee. The constructive criticisms and assistance provided by the above five members of the program of study committee are highly respected.

Dr. Edwin C. Berry is to be thanked for his advice and assistance in carrying out the field studies. Appreciation is extended to Ms. Mary L. Lodholz and Ms. Elva M. Olson from the USDA Corn Insects Research Unit for helping maintain the European corn borer colonies and preparing materials for the field and laboratory. The technical assistance received from Mr. Robert D. Gunnarson, Mr. Roger D. Hull, Mr. Mark T. Vandenberg and Mr. Timothy B. Johnson was much appreciated.

The author wishes to thank Iowa State University and the USDA, SEA, AR for providing the facilities and financial support necessary to make this research possible.

The patience and support provided by my wife, Annie and my son, Jason, during these graduate studies will always be honored and remembered.